STABILIZATION OF LIPID MEMBRANES WITH DENDRITIC POLYMERS

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ABSTRACT

Ion channels incorporated into lipid bilayers can be used as chemical sensors; however, the lack of stability of these bilayers prevents their use in practical sensor devices. In this study, dendrimers were used to stabilize lipid membranes by making use of their high surface functionality and the capability of modifying that functionality. Silica surfaces were coated with sub-monolayers of dendritic polymers, followed by a lipid solution containing a small percentage of fluorescent lipid. The lipid was allowed to incubate on the surface, and then unbound lipid was rinsed off with water. Fluorescence microscopy and fluorometry were used to assess the lipid adsorption to the substrate surfaces. Amine-based dendritic polymers, including polyamidoamine and polyethyleneimines, stabilized the lipids the greatest amount due to positive interactions between the lipid head group and the amine dendrimer. Neutral zwitterionic lipids, such as phosphocholine, were stabilized to a greater extent than negatively charged lipids, probably because the latter were repelled from the negatively charged silica surface.

1. INTRODUCTION

Bilayer lipid membranes (BLM) with incorporated ion channels have been proposed as surfaces for sensing of analytes, such as ions and proteins (Bayley and Cremer, 2001). BLMs have been prepared using a number of different methods, including Langmuir-Blodgett (Takamato, *et al.*, 2001) and solution techniques (Johnson, *et al.*, 2002). However, BLMs are too unstable to be used to make effective and practical sensor devices (Bayley and Cremer, 2001).

Dendrimers are good candidates for stabilizing lipid membranes on solid surfaces because they can be powerful tools in tailoring surface properties (Tully and Frechet, 2001). Dendrimers have high levels of functionality and can be completely or partially modified to enable them to effectively attach to any surface. In addition, dendrimers have been shown to stabilize polyanions (He, *et al.*, 1999), and could therefore potentially stabilize zwitterionic and anionic lipids. As a result, we have conceptualized a fully integrated real-time nano-scale sensor platform for electronic biomolecule sensing and signal processing (Fig. 1). We have also begun efforts in prototyping the underlying nano-electronic architecture (Fig. 2).

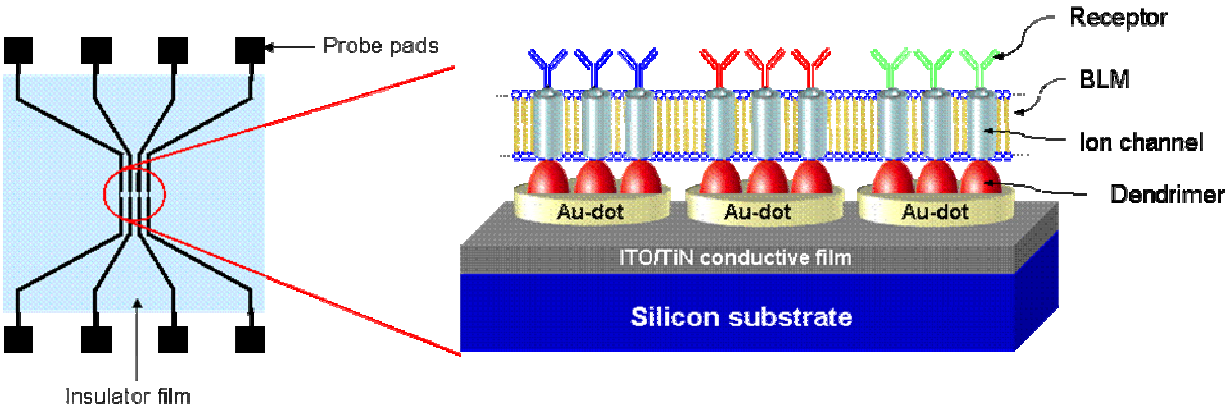

Figure 1: Concept for a fully integrated real-time nano-scale sensor platform for electronic biomolecule sensing and signal processing.

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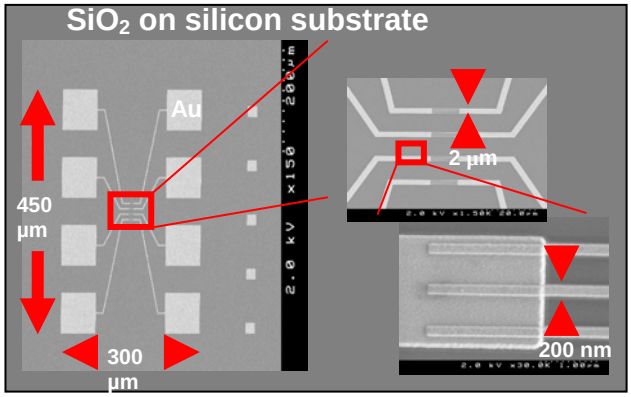

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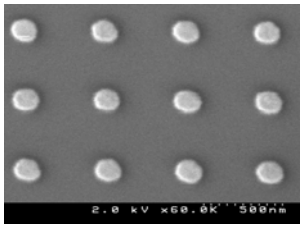

Figure 2: Prototyped nano-electronic architecture of the biochemical sensor device.

This work examines the abilities of dendrimers to stabilize lipid membranes. Various dendrimers and hyperbranched polymers were evaluated. In addition, lipids with different head groups were used to probe the underlying physical chemistry of stabilization.

2. EXPERIMENTAL

2.1 Preparation of Dendritic Polymers

A number of dendritic polymers having various molecular weights and levels of functionality were used in this work (Table 1). Polyamidoamine (PAMAM) dendrimers were purchased from Sigma-Aldrich. The hyperbranched polyethyleneimines Lupasol WF and P (BASF) were also used, along with the linear polyethyleneimine Lupasol LU 321. The hyperbranched polyester, Boltorn H50 (Perstorp), was also used. Solution concentrations of 10^{-5} w/w, 10^{-6} w/w, and 10^{-7} w/w dendrimer in 0.005 M HCl were made. The polyester sample was insoluble in water, so solutions in methanol were made instead at the same concentrations.

2.2 Substrate Preparation

Silicon wafers with a native oxide surface and glass cover slips with SiO_2 surfaces were cleaned in a 50/50 H_2O_2 (30% aq.)/sulfuric acid mixture. Individual wafers were removed from the cleaning solution and rinsed with DI water. The substrate was then placed on the spin coater and spun at 2000 rpm for 48 seconds while again rinsing with DI water. The substrate was flooded with the dendrimer solution, and was allowed to stand for 1 min or 5 min. The sample was spun dry at 2000 rpm for 78 seconds.

Table 1: Molecular weight of dendritic polymers used in this work

Polymer	MW (g/mol)	Functionality	Functionality
PAMAM 2	3.3×10^{3}	1° Amine	16
PAMAM 3.5	1.2×10^{4}	Na^{+} Carboxylate	64
PAMAM 4.5	2.5×10^{4}	Na^{+} Carboxylate	128
PAMAM 5	2.9×10^{4}	1° Amine	128
PAMAM 7	1.2×10^{5}	1° Amine	512
Lupasol WF	2.5×10^{4}	1° and 2° Amine	350
Lupasol P	7.5×10^{5}	1° and 2° Amine	1.1×10^{4}
Lupasol LU321	1×10^{6}	1° Amine	N.A.
Boltorn H50	1.5×10^{4}	Hydroxyl	128

2.3 BLM Preparation

Four different lipids were used in this work (Fig. 3): 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphocholine (PPC), 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (mPC), 1,2-Diphytanoyl-*sn*-Glycero-3-Phosphate [Na salt] (PA), and 1,2-Diphytanoyl-*sn*-Glycero-3-[Phospho-L-Serine] [Na salt] (PS). PC (i.e. PPC and mPC) lipids are zwitterionic and have a net neutral charge, while the zwitterionic PS has a net -1 charge and PA has a charge of -1. 1,2 -Dipalmitoyl-*sn*-Glycero-3-Phosphoethanolamine-N-(7-nitro-2-1,3–benzoxzdiazol-4-yl) (NBD) in chloroform solution was used as the fluorescent lipid tracer at 2 wt% of the basis lipid in all solutions. All lipids were purchased from Avanti.

Lipid membranes were prepared using a solution method (Johnson *et al.*, 2002). The basis lipid was mixed with 2 wt% NBD in chloroform solution to give a final concentration of 1 mg/mL followed by solvent evaporation under nitrogen. The dried lipids were hydrated in 4 ml of deionized water. The mixture was bath sonicated at 45 °C and then tip-sonicated for 10 min to produce small unilamellar vesicles. The samples were centrifuged to remove titanium particles released into the solution from the tip sonicator, and then filtered using a 0.1 µm filter.

Drops of lipid vesicle solutions (20 µL) were placed on clean silica surfaces and the surfaces of silica coated with dendrimers. The samples were allowed to incubate in Petri dishes for 1 hr at room temperature. DI water (4 mL) was added to each of the Petri dishes. After 5 min, the solution from the Petri dish was transferred to a 20 mL vial. The sample was then rinsed with an additional 4 mL DI water. The water washes were tip-sonicated for 5 min in the screw-cap vial to homogenize the samples before characterizing via fluorometry.

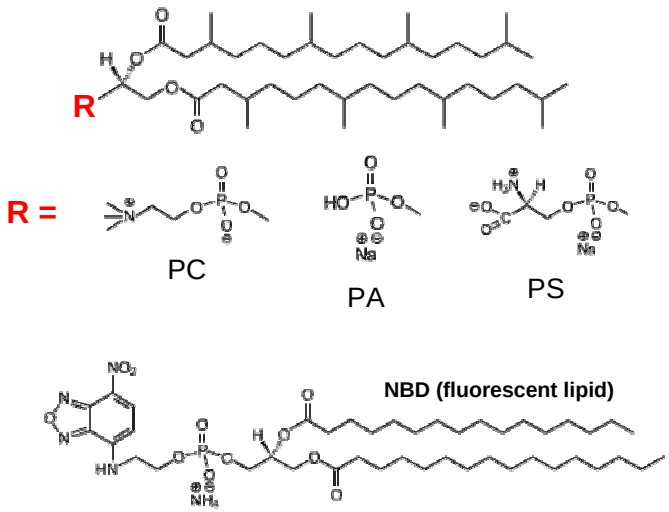

Figure 3: Molecular structures of the lipids used in this work.

The substrates were then transferred to clean glass Petri dishes. Chloroform (4 mL) was added to the dishes and allowed to stand for 2 minutes to remove all adsorbed lipids from the substrate surface. The chloroform wash was transferred to a 20 mL screw-cap vial and then to a cuvette for fluorescence analysis. The chloroform wash was omitted for the samples prepared for fluorescence microscopy.

2.4 Atomic Force Microscopy

Atomic force microscopy was used to examine the surface coverage of dendrimers on the silica substrates. A Digital Instruments Dimension 3100 was used in tapping mode with a silicon nitride tip resonating at 300 kHz. The dendrimers were 1.0-3.0 nm tall and 40-200 nm wide (Fig. 4). These results indicate that polymer spots are approximately 1 dendrimer tall, but are agglomerates of a few dendrimer along the substrate. Sub-monolayer coverage was possible using the spin-coating procedure for concentrations of 10^{-5} w/w dendrimers in solution or lower. Higher coverages are not desired because they would likely interfere with electrical measurements. Concentrations as low as 10^{-7} w/w resulted in very low coverage of dendrimers; therefore, lower concentrations were not used.

2.5 Fluorometry

A Spex Fluoromax-3 Fluorometer was used to measure the fluorescence intensity of the lipid solutions. NBD fluoresces at 520-540 nm; therefore, an excitation wavelength of 470 nm was used with emission spectra of 490-600 nm. Band widths of 5 nm were used with a 2 nm interval and 0.1 s integration time.

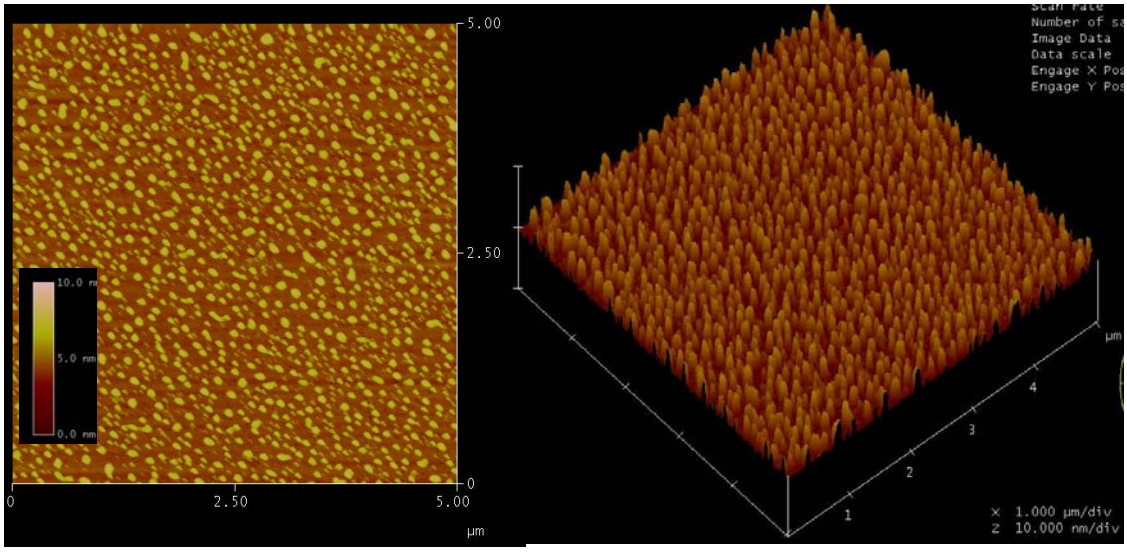

Figure 4: AFM image of PAMAM 7 on silica self-assembled for 1 minute prior to spin-coated from a 10^{-5} w/w solution of 0.005 M HCl.

The water wash was analyzed in polystyrene cuvettes, while the chloroform wash was analyzed in the quartz cuvettes. The quartz cuvettes were rinsed with chloroform, then acetone, followed by copious amounts of deionized water. The polystyrene cuvettes were cleaned with copious amounts of DI water.

An Olympus IX70 fluorescence microscope with Spot RT CCD camera (Diagnostic Instruments, Inc.) was used to examine glass cover slips with attached BLMs. An excitation filter of 460-490 nm with a dichroic mirror of 500 nm and a barrier filter of 520 nm was used. The fluorescence images were captured at 20x optics.

3. RESULTS and DISCUSSION

The water washes removed all non-bound lipids from the substrate surface, and the chloroform wash removed all of the remaining lipids that were bound to the substrate. The fluorescence intensity of the chloroform solution, therefore, is a measure of the amount of lipids bound to the substrate surface and potentially proportional to the BLM stability. In general, we found that dendrimers enhanced the adsorption of lipids. For example, Fig. 5 shows the fluorescence spectra of the lipids in the chloroform wash on SiO_2 substrates with and without dendrimers. The fluorescence intensity was considerably higher for the sample with dendrimer. This indicates that dendrimers either increased the BLM adsorption rate onto the surface or they increased the binding strength of lipids onto the surface. Although this does not necessarily translate into increased BLM stability, increased adsorption is necessary for our hypothesis.

The dendritic polymer itself had a large effect on the adsorption characteristics of the lipids (Fig. 6). Fluorometry and fluorescence microscopy showed that silica coated with PAMAM generations 5 and 7 and Lupasol WF and P had high fluorescence intensity relative to the control samples with no dendrimers on the surface. PAMAM 2 dendrimers gave low fluorescence intensity, but it was slightly higher than that of the control. Although it is possible that PAMAM 2 dendrimers did not stick to the silica surface as well as higher generations, it is likely that dendrimer size plays a role in lipid adsorption. We hypothesize that the highly negative silica surface repels the negative and zwitterionic lipids. As the dendrimer generation increases, the size of the dendrimer increases allowing a larger percentage of functional groups to interact with the lipids rather than the substrate surface, shielding the lipids from the silica surface. Furthermore, the lack of surface amine functionality on PAMAM half-generations (e.g. PAMAM 4.5) caused the fluorescence intensity to be low because the negatively charged surface of these dendrimers repels the lipids and inhibits the formation of lipid membranes.

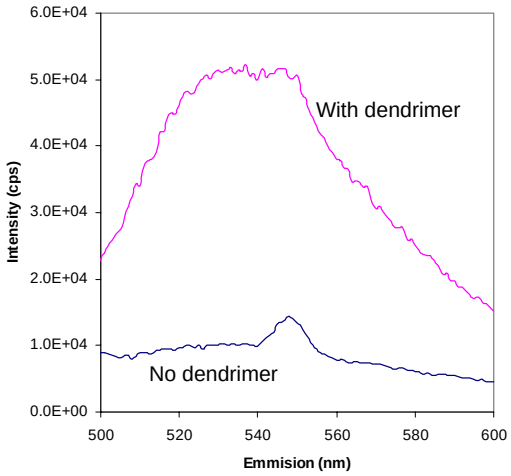

Figure 5: The fluorescence intensity of lipids recovered in the chloroform wash for silica coated with PAMAM 7 dendrimer, relative to plain silica.

The samples coated with the polyester hyperbranched polymer had no significant fluorescence intensity (Fig. 6). This indicates that the chemical functionality of amine dendrimers have specific attractive interactions with the lipids that polyesters do not. It is possible that the amine head groups of PC and PS have attractive interactions, such as hydrogen bonding, directly with the amine dendrimers or indirectly through water.

Samples coated with the linear polyethyleneimine had lower fluorescence intensity than samples coated with hyperbranched Lupasols (Fig. 6). This was expected because the functional groups are more accessible in dendritic polymers than in linear polymers (Tully and Frechet, 2001). Therefore, there is an advantage to using dendritic polymers for stabilizing BLMs due to their high levels and accessibility of surface functionality.

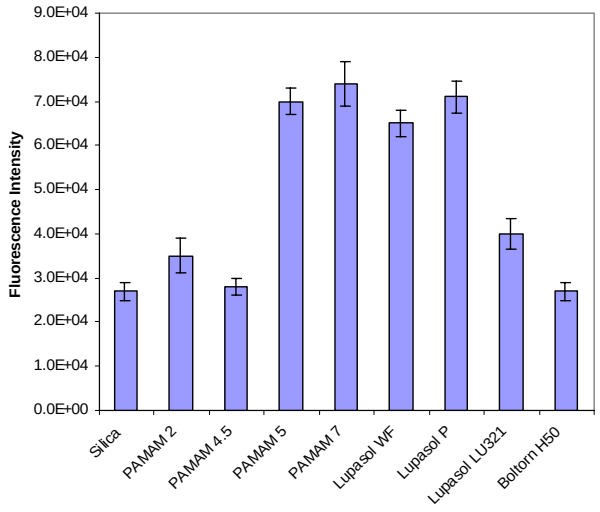

Figure 6: The fluorescence intensity of mPC lipids removed using a chloroform wash from silica surfaces with different polymer coatings.

The fluorescence intensity increased as coverage time before spin coating increased and as the dendrimer solution concentration increased (Fig. 7). AFM measurements showed that surface coverage increased with these parameters. This further indicates that increased dendrimer coverage enhanced the adsorption of lipids to the substrate.

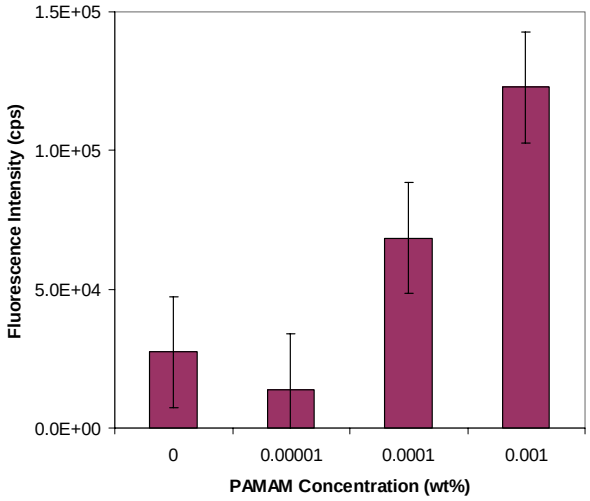

Figure 7: Fluorescence intensity as a function of the PAMAM concentration used in the spin coating process. Dendrimers were allowed to self-assemble on the surface for 1 minute prior to spinning dry.

The particular lipids used had a large effect on the surface coverage. Fig. 8a shows that PC lipids had uniform surface coverage. When comparing to the control samples (i.e. no dendrimers on the surface – Fig. 8b), it is clear that dendrimers enhanced the adsorption of PC lipids to the surface. PS had a fairly high surface coverage of lipids (Fig. 9a). However, unlike PC, the surface coverage was very spotty, showing that the attached vesicles remained intact. Therefore, PS did not form a smooth BLM on SiO_2 surfaces. PA adsorbed poorly to the surface, and only showed low fluorescence intensity (Fig. 9b).

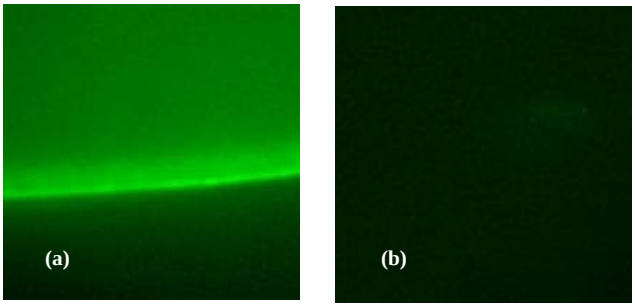

Figure 8: Fluorescence images after rinsing with water of (a) mPC lipids on glass coated with PAMAM 7 (0.0001 w/w) relative to (b) mPC coated on plain glass. The image in (a) shows the contrast between the regions coated with the lipid solution (green) and the region not coated (dark).

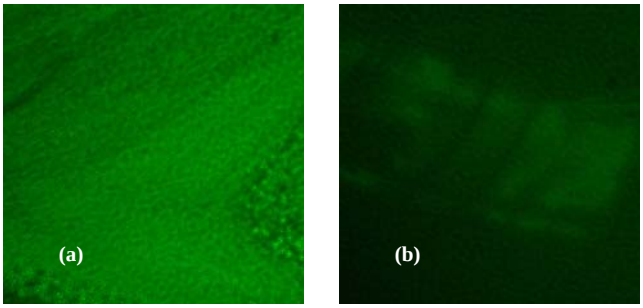

Figure 9: Fluorescence images of (a) PS lipids and (b) PA lipids on glass coated with PAMAM 7 (0.0001 w/w).

Silica surfaces in water have a net negative charge. Therefore, it seems likely that negatively charged lipids are somewhat repelled by the surface. In fact, the PA has only a single negative charge and appeared to be more repelled by the surface than the other lipids. On the other hand, the negatively charged zwitterionic PS attaches to the surface because of the presence of the positive charge on the lipid. It is likely that the net negative charge prevents the lipids from maximizing their contact with the surface. Therefore, the vesicles would remain intact on the surface. In addition, this result offers further evidence that the amine head groups of PC and PS allow for attractive interactions between these lipids and amine dendrimers.

4. CONCLUSIONS

BLMs with incorporated ions can be used as the basis of a chemical sensor device. BLMs can be stabilized in the presence of an inorganic substrate by adhering to dendritic polymers coated on the substrates. Amine terminated dendrimers and hyperbranched polymers enhanced the adhesion/stability of zwitterionic lipids to the greatest extent. Increasing the dendrimer coverage also increased the BLM stability. Therefore, dendrimers can indeed be used to help stabilize BLMs to enable their use in a chemical sensor device.

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